

Effects of Exogenous Nitrogen Source Application in Cd-Contaminated Vegetable Fields: Post-print

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Abstract

This study investigated the effects of external nitrogen sources on cadmium (Cd) uptake by different leafy vegetables and soil Cd availability in Cd-contaminated vegetable fields, aiming to clarify the influence of nitrogen application on soil Cd and to comprehensively evaluate the application effects of different nitrogen sources, thereby providing a reference for the rational utilization of nitrogen fertilizers to reduce Cd content in leafy vegetables. In a Cd-contaminated vegetable field with a Cd content of $0.628 \text{ mg} \cdot \text{kg}^{-1}$, the effects of four nitrogen fertilizers (urea, calcium nitrate, ammonium nitrate, and ammonium bicarbonate) at a nitrogen application rate of $150 \text{ kg} \cdot \text{hm}^{-2}$ on Cd content, quality, and soil Cd availability of Aijiaokuishan black leaf Chinese cabbage (*Brassica chinensis* L.) and white-stem sharp-leaf amaranth (*Amaranthus mangostanus* L.) were examined. The results showed that under field conditions, compared with the no-nitrogen control treatment, all four nitrogen fertilizers increased the yields of the two leafy vegetables to varying degrees and reduced their shoot and root Cd contents. Among the four nitrogen fertilizers, urea exhibited the best yield-increasing effect on white-stem sharp-leaf amaranth, with a yield increase of 47.5%; ammonium bicarbonate showed the best yield-increasing effect on Aijiaokuishan black leaf Chinese cabbage, with an increase of 59.7%. Calcium nitrate was superior to other nitrogen fertilizers in reducing shoot and root Cd contents in both vegetables; compared with the control, the shoot and root Cd contents of white-stem sharp-leaf amaranth treated with calcium nitrate decreased by 41.6% and 24.1%, respectively, while those of Aijiaokuishan black leaf Chinese cabbage decreased by 32.2% and 25.9%, respectively. The four nitrogen sources had different effects on total Cd uptake in shoots, NO_3^- -N, NO_2^- -N, vitamin C, and soluble sugar contents of the two leafy vegetables, and also differentially influenced soil pH and DTPA-Cd content. Specifically, the ammonium nitrate treatment decreased soil pH by 0.12 and 0.25 units for the

two vegetables compared with the control, while soil DTPA-Cd content significantly increased by 15.3% and 14.6%, respectively; the ammonium bicarbonate treatment showed opposite trends. The comprehensive evaluation results revealed that the comprehensive weighted average values of all four nitrogen fertilizers were higher than that of the control, with calcium nitrate being the relatively highest, indicating that calcium nitrate had the relatively best comprehensive application effect in Cd-contaminated vegetable fields. Therefore, calcium nitrate can be used as a preferred nitrogen source in Cd-contaminated soils.

Full Text

Preamble

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Effects of Nitrogen Addition on Above-Standard Cd-Contaminated Soils in Vegetable Fields*

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Abstract

This study investigated the effects of nitrogen application on cadmium (Cd) uptake by different leafy vegetables and soil available Cd content in above-standard Cd-contaminated vegetable soils. The objective was to comprehensively evaluate different nitrogen sources and provide guidance for utilizing nitrogen fertilizers to reduce Cd concentrations in leafy vegetables. In a Cd-contaminated vegetable field with total Cd content of $0.628 \text{ mg} \cdot \text{kg}^{-1}$, a field experiment examined the effects of four nitrogen fertilizers—urea, calcium nitrate, ammonium nitrate, and ammonium bicarbonate—applied at $150 \text{ kg} \cdot \text{hm}^{-2}$ on yield, quality, Cd content, and nitrogen content of two leafy vegetables: *Brassica chinensis* L. (BC) and *Amaranthus mangostanus* L. (AM). Soil DTPA-Cd content, pH, and comprehensive effects of nitrogen addition were also investigated. The results showed that all nitrogen sources increased yields and decreased Cd contents in both shoot and root systems of the two leafy vegetables. The highest increase in BC yield (59.7%) was caused by NH_4HCO_3 treatment, while the largest increase in AM yield (47.5%) was caused by $\text{CO}(\text{NH}_2)_2$ treatment, compared with the control. However, the lowest Cd contents in both vegetables were observed under $\text{Ca}(\text{NO}_3)_2$ treatment among all nitrogen treatments. $\text{Ca}(\text{NO}_3)_2$ decreased Cd content by 41.6% and 24.2% in AM shoot and root, respectively, and by

32.2% and 25.9% in BC shoot and root, respectively. Moreover, the total content of Cd, nitrate, nitrite, Vitamin C, and soluble sugar in the shoot system varied with different nitrogen resources, as did changes in soil pH and DTPA-Cd content. NH_4NO_3 application decreased soil pH by 0.12 and 0.25 units and increased soil DTPA-Cd content by 15.3% and 14.6% for AM and BC, respectively. The reverse trend was noted under NH_4HCO_3 treatment. Comprehensive evaluation results showed that the four nitrogen resources had much higher synthetic weighted values than the control. Among all treatments, the highest value was observed in $\text{Ca}(\text{NO}_3)_2$ treatment, suggesting that $\text{Ca}(\text{NO}_3)_2$ had the best comprehensive application effect in above-standard Cd-contaminated soils. Thus, $\text{Ca}(\text{NO}_3)_2$ could be used as an optimum nitrogen source in above-standard Cd-contaminated soils in vegetable fields.

Keywords: Nitrogen source; Vegetable field; Above-standard Cd-contaminated soil; Leafy vegetable; Availability of soil Cd

Introduction

In recent years, industrial development, mining activities, atmospheric deposition, and improper agricultural practices have caused heavy metal concentrations in the Pearl River Delta region to rise, with cadmium (Cd) contamination in vegetable field soils becoming particularly prominent. Leafy vegetables exhibit the strongest Cd absorption capacity and accumulation among crops, posing the most serious risk of Cd exceeding food safety standards. As one of the most toxic heavy metals, long-term consumption of Cd-contaminated food poses potential health hazards, necessitating measures to reduce Cd content in edible parts of leafy vegetables to mitigate food chain risks.

Fertilization represents one of the most important yield-increasing measures in agricultural production and a critical pathway for material input in farmland ecosystems. Inorganic nitrogen fertilizers are chemical crystals that are relatively clean and generally do not cause heavy metal contamination of soils. However, the acidity or alkalinity of nitrogen fertilizers and the acids or bases produced during their transformation can significantly affect rhizosphere soil pH, which in turn has the greatest influence on soil Cd solubility. Additionally, complexation of Cd by nitrogen fertilizer components and cation exchange of Cd on soil colloids mean that nitrogen application affects Cd bioavailability in soil and subsequently plant Cd uptake. Due to differences in experimental soils and crops, the effects of the same nitrogen fertilizer on heavy metal bioavailability and plant uptake can vary substantially. Maier et al. found that in alkaline light-textured soils, crops fertilized with $\text{Ca}(\text{NO}_3)_2$ had higher Cd content than those receiving $(\text{NH}_4)_2\text{SO}_4$, due to exchange between Ca^{2+} and soil-adsorbed Cd^{2+} . Alpha et al. concluded that $(\text{NH}_4)_2\text{SO}_4$ application resulted in greater plant Cd uptake than $\text{Ca}(\text{NO}_3)_2$, primarily because H^+ secretion during NH_4^+ -N absorption or nitrification of NH_4^+ -N decreased rhizosphere soil pH. Current research on ni-

trogen fertilizer effects on heavy metal uptake focuses on two aspects: reducing plant heavy metal uptake through rational nitrogen application, or increasing uptake to enhance phytoremediation efficiency. While these studies have clarified mechanisms of different nitrogen fertilizers on heavy metals to some extent, most results were obtained under laboratory conditions, and their effectiveness under field conditions with numerous environmental factors remains uncertain. Therefore, this study conducted a field plot experiment in a Cd-contaminated vegetable field to investigate the effects of different nitrogen forms on yield, quality, and soil quality of two leafy vegetables with different Cd accumulation characteristics, further clarifying the practical application value of nitrogen fertilizers in reducing vegetable Cd content and providing a scientific basis for safe vegetable production in Cd-contaminated soils through rational nitrogen management.

1.1 Experimental Site Description

The field experiment was conducted in the suburbs of Guangzhou, located in the core area of the Pearl River Delta, where leafy vegetables are the main crops. The region features a south subtropical monsoon climate characterized by warm, rainy conditions with abundant light and heat, long summers, and short frost periods. Annual rainfall and heat occur simultaneously, with abundant precipitation averaging 1,982.7 mm annually and a mean annual temperature of 22°C.

The experimental field, used for continuous leafy vegetable production, had the following soil physicochemical properties: pH 6.8, organic matter 36.9 g · kg⁻¹, total nitrogen 2.39 g · kg⁻¹, alkaline hydrolyzable nitrogen 230 mg · kg⁻¹, available phosphorus 270 mg · kg⁻¹, readily available potassium 310 mg · kg⁻¹, total Cd 0.628 mg · kg⁻¹, and DTPA-Cd 0.224 mg · kg⁻¹. The soil was neutral, with total nitrogen, available nitrogen, available phosphorus, and readily available potassium all at extremely abundant levels. According to the Soil Environmental Quality Standard (GB15618–1995), the soil Cd content exceeded the secondary standard, classifying it as a Cd-contaminated vegetable field.

1.2.1 Vegetable Varieties

Based on preliminary research results, two leafy vegetables with different Cd accumulation characteristics were selected: *Brassica chinensis* L. (a low-Cd-accumulating vegetable) and *Amaranthus mangostanus* L. (a high-Cd-accumulating vegetable). Seeds were purchased from local markets.

1.2.2 Fertilizer Types

Urea (N 46%) and ammonium bicarbonate (N 17%) were purchased from local agricultural supply markets. Calcium nitrate (N 11.8%), ammonium nitrate (N 32%), and potassium sulfate (K₂O 50%) were industrial-grade products purchased from a chemical market in Guangzhou. Calcium dihydrogen phosphate

(P O 55%) was also used.

1.3.1 Experimental Design and Layout

Both vegetable species received four nitrogen treatments: urea, calcium nitrate, ammonium nitrate, and ammonium bicarbonate, each applied at $150 \text{ kg(N)} \cdot \text{hm}^{-2}$. A no-nitrogen control was designated as N0. All treatments received identical phosphorus (calcium dihydrogen phosphate) and potassium (potassium sulfate) applications at rates of $60 \text{ kg} \cdot \text{hm}^{-2}$ P O and $112.5 \text{ kg} \cdot \text{hm}^{-2}$ K O, respectively.

1.3.2 Field Management

Both *Brassica chinensis* and *Amaranthus mangostanus* were directly seeded without basal fertilizer. Following local fertilization practices, nitrogen, phosphorus, and potassium were applied as topdressing in three splits at ratios of 30%, 35%, and 35% of the total designed amounts throughout the growth period. Each treatment was replicated four times. Plot size was 20.6 m^2 for both vegetables, with randomized field arrangement and consistent field management practices across all plots.

1.3.3 Sample Collection and Measurement

Before sowing, soil samples from the 0-20 cm layer were collected to determine basic physicochemical properties. Plant samples: At harvest, all plants in each plot were collected to determine marketable yield. From each plot, 2 kg of fresh samples were randomly selected, and 30 plants of each species were randomly uprooted to collect root systems. Samples were transported to the laboratory, washed with tap water and deionized water, soaked in $0.5 \text{ mmol} \cdot \text{L}^{-1}$ CaCl solution for 30 minutes, rinsed again with deionized water, surface moisture absorbed with filter paper, and then homogenized for Cd content determination. Soil samples: After harvest of both vegetables, soil cores were taken from 10 random points in each plot at 0-20 cm depth, mixed to form one composite sample per plot, air-dried at room temperature, and passed through a 20-mesh sieve for analysis.

1.4 Measurement Items and Methods

Plant Cd content was determined by graphite furnace atomic absorption spectrophotometry after HNO₃-HClO₄ digestion. Vitamin C was measured by 2,6-dichloroindophenol titration, soluble sugar by anthrone colorimetry, nitrate by UV spectrophotometry, and nitrite by the naphthylethylenediamine hydrochloride method. Soil pH was determined by potentiometry (water:soil = 2.5:1), and available Cd was extracted with DTPA and measured by flame atomic absorption spectrophotometry.

1.5 Data Processing

Data were organized using Microsoft Excel, and statistical analysis including ANOVA was performed using SAS 8.1 software.

Results

2.1 Effects of Different Nitrogen Sources on Leafy Vegetable Yield

The four nitrogen fertilizers significantly affected growth and yield of both vegetables (Table 1). In the no-nitrogen treatment, despite high alkaline hydrolyzable nitrogen content of $230 \text{ mg} \cdot \text{kg}^{-1}$ in the pre-planting soil (indicating abundant nitrogen), both *Amaranthus mangostanus* and *Brassica chinensis* showed obvious nitrogen deficiency symptoms with light green color and suppressed aboveground growth, resulting in lower yields than all nitrogen treatments.

For *Amaranthus mangostanus*, urea treatment produced the highest yield, increasing by 47.5% compared with the control, followed by calcium nitrate with a 30.9% increase. Ammonium nitrate and ammonium bicarbonate showed weaker yield-increasing effects with no significant differences from the no-nitrogen treatment. For *Brassica chinensis*, all four nitrogen fertilizers significantly increased yield, with ammonium bicarbonate showing the best effect (47.7% increase), followed by ammonium nitrate (32.7% increase). Two-way ANOVA indicated that both vegetable species ($F = 212.6$) and nitrogen sources ($F = 9.06$) had significant effects on shoot and root Cd content, but their interaction ($F = 2.29$) was not significant.

Vegetable species and nitrogen source both significantly affected total Cd uptake in shoots, with a significant interaction between them (Table 1). Compared with the control, calcium nitrate, ammonium nitrate, and ammonium bicarbonate all reduced total Cd uptake in *Amaranthus mangostanus* shoots, with calcium nitrate showing the greatest reduction (24.0%). In contrast, urea significantly increased total Cd uptake in *Amaranthus mangostanus* shoots by 18.8%. All four nitrogen fertilizers significantly increased total Cd uptake in *Brassica chinensis* shoots, with increases ranging from 25.01% to 51.2%.

2.2 Effects of Different Nitrogen Sources on Shoot and Root Cd Content

Two-way ANOVA showed that both vegetable species and nitrogen sources significantly affected shoot and root Cd content, but their interaction was not significant. In all treatments, shoot Cd content in both vegetables was below China's food Cd limit standard [$0.2 \text{ mg} \cdot \text{kg}^{-1}$ (fresh weight)]. However, application of different nitrogen sources significantly reduced shoot Cd content compared with the no-nitrogen control (Table 2). For *Amaranthus mangostanus*, all four nitrogen sources reduced shoot Cd content, with calcium nitrate showing the best effect (41.6% reduction), followed by ammonium nitrate (27.8% reduction). Ammonium bicarbonate and urea showed weaker Cd-reducing effects, decreasing by

20.5% and 19.5%, respectively. For *Brassica chinensis*, all four nitrogen sources significantly reduced shoot Cd content by 29.9%–32.2%, with no significant differences among nitrogen treatments. Under the same nitrogen treatment, shoot Cd content in *Amaranthus mangostanus* was significantly higher than in *Brassica chinensis*, consistent with their Cd accumulation characteristics—*Amaranthus mangostanus* (amaranth genus) is a high-Cd-accumulating species, while *Brassica chinensis* (brassica genus) is a low-Cd-accumulating species.

Compared with the control, the four nitrogen sources also reduced root Cd content to varying degrees. For *Amaranthus mangostanus*, all nitrogen sources except ammonium nitrate significantly reduced root Cd content, with urea and calcium nitrate showing the best effects (both 24.1% reduction). For *Brassica chinensis*, urea and calcium nitrate were most effective in reducing root Cd content, with reductions of 20.3% and 25.9%, respectively, while ammonium nitrate and ammonium bicarbonate showed no significant Cd-reducing effects.

2.3 Effects of Different Nitrogen Sources on Shoot NO₃⁻-N and NO₂⁻-N Content

Two-way ANOVA indicated that vegetable species, nitrogen source, and their interaction all significantly affected shoot NO₃⁻-N content. All four nitrogen fertilizers promoted NO₃⁻-N accumulation in both vegetables (Table 3). For *Amaranthus mangostanus*, shoot NO₃⁻-N content in the four nitrogen treatments increased by 3.03–3.96 times compared with the unfertilized control, with urea showing the greatest increase and ammonium bicarbonate the smallest. For *Brassica chinensis*, shoot NO₃⁻-N content increased by 6.60–7.03 times, with no significant differences among nitrogen treatments. Under the same nitrogen treatment, shoot NO₃⁻-N content in *Brassica chinensis* was much higher than in *Amaranthus mangostanus*, possibly due to species differences.

The four nitrogen fertilizers had significantly different effects on shoot NO₂⁻-N content. Compared with the control, calcium nitrate did not significantly increase NO₂⁻-N content in *Amaranthus mangostanus*, while the other three nitrogen sources significantly increased it by 1.48–2.71 times. Calcium nitrate significantly increased NO₂⁻-N content in *Brassica chinensis*, while urea had no significant effect, and ammonium nitrate and ammonium bicarbonate decreased it, with ammonium bicarbonate showing a significant reduction. However, shoot NO₂⁻-N content in both vegetables did not exceed the standard limit of 4 mg·kg⁻¹ (as NaNO₂) specified in the “Limits of Contaminants in Foods.” The maximum NO₂⁻-N content among all fertilization treatments was 0.78 mg·kg⁻¹, indicating no health pollution risk.

2.4 Effects of Different Nitrogen Sources on Shoot Vitamin C and Soluble Sugar Content

Two-way ANOVA showed that vegetable species, nitrogen source, and their interaction all significantly affected shoot vitamin C and soluble sugar content.

Nitrogen application had different effects on vitamin C and soluble sugar content in the two vegetables (Table 4). For *Brassica chinensis*, all nitrogen treatments significantly reduced shoot vitamin C and soluble sugar content compared with the no-nitrogen control. Vitamin C content was lowest under urea treatment (37.7% reduction), while soluble sugar content was lowest under ammonium bicarbonate treatment (69.4% reduction). *Amaranthus mangostanus* showed different trends: urea and calcium nitrate increased shoot vitamin C content, while ammonium bicarbonate increased soluble sugar content (though not significantly compared with the control). The other three nitrogen sources reduced soluble sugar content in *Amaranthus mangostanus*, but differences from the control were not significant.

2.5 Effects of Different Nitrogen Sources on Soil Available Cd Content and pH

Two-way ANOVA indicated that vegetable species and nitrogen source significantly affected soil DTPA-Cd content, while their interaction was not significant. Nitrogen source significantly affected soil pH, but vegetable species and their interaction did not. Nitrogen fertilizers influenced soil pH to some extent. Among all treatments, ammonium nitrate resulted in the lowest soil pH, with a significant reduction compared with the no-nitrogen control in soil planted with *Brassica chinensis*. Ammonium bicarbonate treatment produced the highest soil pH, though not significantly different from the control.

Different nitrogen fertilizers had varying effects on soil DTPA-Cd content (Table 5). For soil planted with *Amaranthus mangostanus*, all nitrogen fertilizers except calcium nitrate increased soil DTPA-Cd content to varying degrees, with ammonium nitrate showing a significant increase compared with the no-nitrogen control. For soil planted with *Brassica chinensis*, ammonium nitrate and urea increased soil DTPA-Cd content compared with the control, with ammonium nitrate showing a significant difference. Calcium nitrate and ammonium bicarbonate reduced soil DTPA-Cd content, but differences from the control were not significant. Under the same treatment, soil DTPA-Cd content differed between the two vegetable species, with soil planted with *Amaranthus mangostanus* showing higher DTPA-Cd content than soil planted with *Brassica chinensis*, possibly due to the Cd-activating effect of amaranth.

2.6 Comprehensive Evaluation of Four Nitrogen Sources

To comprehensively evaluate the application effects of the four nitrogen fertilizers in Cd-contaminated vegetable soils, a multi-criteria assessment was conducted using the following indicators: vegetable yield, shoot and root Cd content, total shoot Cd uptake, shoot NO⁻N, vitamin C, and soluble sugar content, as well as soil DTPA-Cd content and pH. Among these, vegetable yield, total Cd uptake, vitamin C content, soluble sugar content, and soil pH were maximization indicators (higher values preferred), while the others were minimization indicators (lower values preferred). Data were first normalized using reciprocal

transformation for minimization indicators, then dimensionless, and finally evaluated using principal component analysis to rank the comprehensive effects of the four nitrogen fertilizers. The comprehensive weighted values are shown in Table 6. For *Amaranthus mangostanus*, the ranking was: calcium nitrate > urea > ammonium nitrate > ammonium bicarbonate > control. For *Brassica chinensis*, the ranking was: calcium nitrate > ammonium bicarbonate > urea > ammonium nitrate > control.

Discussion and Conclusion

Previous studies have shown that nitrogen application in Cd-contaminated soils significantly affects plant growth, soil properties, and heavy metal forms and availability, thereby influencing heavy metal translocation in plants. Under the conditions of this field experiment, nitrogen application significantly reduced shoot Cd content in vegetables. For *Brassica chinensis*, shoot Cd content showed a significant negative correlation with biomass (correlation coefficient $r = -0.6336$, $P = 0.0112$), while for *Amaranthus mangostanus*, the negative correlation between shoot Cd content and biomass was not significant (correlation coefficient $r = -0.4474$, $P = 0.0945$), indicating a “dilution effect” that contributed to reduced Cd content in edible parts under nitrogen treatments.

Nitrogen fertilizers also affect heavy metal uptake by altering soil physicochemical properties such as DTPA-Cd content and pH. Zhao Jing reported that acidification from ammonium nitrogen fertilizers could increase soil heavy metal availability; ammonium nitrate reduced soil pH and increased extractable Cd content, with changes in extractable Cd showing a strong negative correlation with pH changes. In this field experiment, the acidifying effect of ammonium nitrate enhanced soil Cd availability. Portmann also concluded that $150 \text{ mg} \cdot \text{kg}^{-1}$ ammonium nitrate promoted wheat growth and increased Cd uptake by reducing soil solution pH, increasing H^+ activity and competition between positively charged H^+ and metal ions for sorption sites on soil minerals and organic matter, thereby releasing more free metal ions into solution. The effects of calcium nitrate and urea on soil pH and DTPA-Cd content were less pronounced than in previous pot experiments, but calcium nitrate treatment resulted in the lowest shoot and root Cd content and total Cd uptake, possibly because added calcium increased competition with Cd for root absorption sites, reducing Cd uptake and translocation. Tu et al. suggested that urea at $200 \text{ mg(N)} \cdot \text{kg}^{-1}$ transformed soil Pb and Cd into less active carbonate-bound and Fe-Mn oxide-bound forms. Other studies found that nitrogen application under high Cd stress promoted poplar growth, possibly related to nitrogen's important role in detoxification processes via glutamate and glutathione pathways.

Nitrogen application increased NO_3^- -N content and reduced vitamin C and soluble sugar content in both vegetables to varying degrees, affecting vegetable safety and nutritional value to some extent. Therefore, comprehensive evaluation of nitrogen fertilizer application effects in Cd-contaminated farmland is crucial for sustainable and safe utilization of contaminated soils and safe agricul-

tural production. Current evaluation standards for contaminated soil remediation in China primarily focus on reducing pollutant concentrations to levels that pose no threat to human health and ecosystems. However, chemical immobilization/stabilization methods only change heavy metal forms without reducing total content, making total heavy metal reduction an inappropriate evaluation criterion that does not truly reflect remediation effectiveness.

This study aimed to regulate Cd-contaminated vegetable fields through fertilization to reduce heavy metal content in agricultural products while achieving high yield and quality and improving soil physicochemical properties. Therefore, a multi-angle evaluation of nitrogen fertilizer effects was attempted, considering vegetable yield, Cd content, quality indicators, and soil properties. The evaluation results showed that calcium nitrate had the highest comprehensive weighted value for both vegetable species, indicating it can be recommended as a preferred nitrogen fertilizer for Cd-contaminated vegetable fields.

Although nitrogen application significantly affects crop growth and Cd accumulation in Cd-contaminated soils, current research on these effects remains insufficient, particularly regarding mechanisms by which nitrogen alleviates Cd stress-induced growth inhibition. Therefore, further research is needed on the mechanisms of nitrogen involvement in plant Cd detoxification under field conditions and on Cd form transformation under N-Cd interactions to elucidate the mechanisms of nitrogen effects on Cd and provide a theoretical basis for rational nitrogen use in Cd-contaminated farmland.

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